## Humoral Immunity *note that this lecture is out of order this year*

Humoral immunity (immunity in blood and body fluids) = B cell antibody response and its influence by helper T cells. Most important for extracellular bacteria and toxins and some viruses.

Definitions

**protein antigen**

a foreign protein that induces production of specific antibodies by the host. Can be directly recognized by B cells (BCR) and indirectly (after processing and presentation of peptide by MHC) by T cells (TCR)

**polysaccharide antigen**

a repeating sugar structure on the surface of a microorganism that can induce an antibody response. Is not recognized by T cells because it is not a polypeptide

**T cell help**

A term referring to signals that T cells provide to B cells to help them make antigen-specific antibodies. You will learn later about the specific T helper subsets that are specialized to help B cells.

**BCR (B cell receptor = surface Ig)**

- Two functional components: the variable component for Ag recognition, and invariant component for signaling.

- Surface Ig is expressed along with **Ig** and **Ig**, which are the signaling components of the "complete" BCR. Only when Ig is secreted does C region have effector function without Ig and Ig.

- B cell activation is initiated by BCR crosslinking (clustering together) (Fig. 9.1)

- most pathogen surfaces have repetitive epitopes that bring together multiple BCR at contact site

Activation of naïve B cells (Fig. 9.2)

- BCR signaling:

Ig and Ighave **ITAM**s (intracellular tyrosine activation motifs) =

BCR crosslinking causes **Src** family tyrosine kinases to phosphorylate ITAMs

**Syk** tyrosine kinase binds to phosphorylated ITAMs and becomes activated; Syk promotes further signaling events leading to initial B cell activation

- B cells require additional signals (besides BCR crosslinking) to become fully activated and secrete antibodies.

- primary BCR signal can be enhanced by co-crosslinking of the **B-cell co-receptor** (Fig. 9.3, 9.5)

= **CD19/CR2**/CD81 complex on B cells (Fig. 9.3); CR2 binds C3d (a breakdown product of C3b) deposited on the pathogen surface near Ag recognized by BCR; enhances Src kinase activation and other signals (Fig. 7.5)

* For B cells that recognize protein antigens, a second distinct signal is provided by helper T cells. Antigens for which T cell help is required for antibody production are called "**thymus-dependent (TD) antigens**". We will return to this below. Some antigens (**thymus-independent (TI) antigens**) have structural characteristics that allow the B cells that bind to them to become activated without any T cell help.

# TI antigens

Many microorganisms have repeating structural features on surface (polysaccharide, proteoglycan, etc.). These can induce some B cells to produce Ab without T cell help.

Two categories: **TI-1** and **TI-2**.

TI-1 provide a second signal themselves by interacting with a second receptor on the B cell that is distinct from the BCR (e.g. lipopolysaccharide (**LPS**) binds **TLR4**; bacterial DNA binds **TLR9**).

(Fig. 9.6)

Rare B cells express Ig that directly recognizes LPS also (left side of diagram), so activation results in secretion of Ab specific for LPS. More common are B cells whose Ig recognizes some other surface feature of bacteria (+/- complement and co-receptor), with LPS/TLR4 interaction providing the second signal (center panel). TLR9 can also provide the second signal (right panel).

TI-2 do not provide a second ligand but produce some response in the B cell because of massive crosslinking of the BCR (e.g. bacteria with repeating polysaccharide coat). (Fig. 9.7)

TI responses are rapid (2-4 days) but mostly IgM and are not associated with SHM/affinity maturation, or with production of memory B cells. Useful as a T-independent early response to infection.

# B Cell Activation, Part I

- B and T interaction: happens in the secondary lymphoid tissues.

From Chapter 6: B cell traffic in the absence or presence of antigen (Figures 6.20, 6.21 and 6.22)

Within lymphoid tissues, B cells and T cells both exit the blood via the high endothelial venule (**HEV**) to enter the T cell area. T cells stay but B cells then migrate to the **follicles**. This is all driven by chemokines (Fig. 6.21). CCL21 and CCL19 attract B and T cells to HEV and drive migration into LN. CXCL13 then recruits B cells to follicles.

If no antigen encountered, B cells exit via the medulla and efferent lymphatics (Fig. 6.20).

If antigen is encountered, clonal selection and differentiation of T and B cells occurs (Fig. 6.22). More detail in chapter 9, described below.

How do antigens get to the areas where B cells can see them?

- Antigens arrive in the lymph node either free or by APCs in the periphery (dendritic cells). Specialized macrophages lining the entry to lymph node from afferent lymph can trap intact antigens on cell surface without processing (not in book; see marked up Figure 6.22). Classical DCs can also present non-processed Ag on surface (not in book; see marked up Figure 6.22).

- T cells and B cells enter the lymph node by extravasation through **HEV**. (Fig. 9.8) or via afferent lymphatic vessels if they have surveyed another lymph node previously.

- Antigen is presented by dendritic cells to T cells in **T cell zone** of lymph node. Antigen-specific T cells are trapped (stop migrating) here and become activated.

- Naïve B cells enter the LN by the same route as T cells (HEV or afferent lymph) and move first through the T cell zone and take residence in the follicles. Those that encounter specific antigen bind to Ag with cell surface Ig (BCR) +/- co-receptor. The B cell endocytoses the Ag/Ab complex, processes it, and presents antigenic peptides on its surface with MHC class II. When the initial Ag recognition by B cells occurs in the T cell zone, the B cells are arrested and do not continue to the follicle. When the initial Ag recognition by B cells occurs in the follicle, they migrate towards the border of the T cell zone to “seek help”. (Fig. 9.8)

- T cell (that has been activated by APC) that is specific to the antigenic peptide+MHC on B cell forms a stable contact with the B cell (Fig. 9.9). This **cognate interaction** accomplishes two things.

1. **CD40** on B cell binds **CD40 ligand (CD40L)** on T cell; CD40 sends activation signals.

2. T cell also secretes cytokines that can activate B cell (e.g. **IL-4**).

- T cells form synapse with cognate B cell for effective delivery of helper cytokines (Fig. 9.10)

- Frequency of a naïve lymphocyte specific for a given antigen is very rare: 1 in 10,000 to 1,000,000. So the chances of a B cell meeting specific helper T, if they were all just circulating in blood, is between 1 in 108 and 1 in 1012. This problem is solved by having B and T cells meet up in lymph nodes, at the border of the T cell zone and B cell follicles.

- Some B cells migrate to the medullary cords (a central area of the lymph node near the efferent lymphatic vessel) to form a “primary focus” and differentiate into **plasma cells** and start secreting antibodies early in the immune response. (Fig. 9.13, left). B cells activated by TI antigens also trigger plasma cell production early in immune response. Sources of early antibodies, mostly IgM isotype.

- Some activated B cells and their helper T cells also migrate to **primary follicles**, which develop into **secondary follicle** (with **germinal centers**). (Fig. 9.13, right). Rapid proliferation of B cells and T cells in germinal center is a reason why draining lymph nodes swell during response to infection

# Germinal Centers (B Cell Activation, Part II)

- Germinal center architecture (Fig. 9.14): Complex structure with proliferating activated B cells, dying B cells, macrophages that clear out the dead B cells, T helper cells, **follicular dendritic cells**. (Fig. 9.12).

FDCs have antigens (in the form of **immune complexes** of Ag with complement fragments) trapped on the cell surface that B cells can bind. FDCs are not derived from hematopoietic stem cells.

FDC is also different from a regular dendritic cell in that they do not express class II MHC, and they do not present antigens this way. They have long extensions that express Fc receptors and complement receptors, and these receptors have immune complexes complexes bound to them. These are retained for a long time, but portions can be plucked off by B cells and internalized along with some FDC membrane. The role of FDC is to be an “antigen depot” for selecting B cells after somatic hypermutation, as well as to provide survival signals for naive B cells.

- If BCR can bind its antigen on FDC, expression of anti-apoptotic proteins are induced and the B cell lives. In addition, higher affinity binding increases internalization of Ag, and presentation of peptides to T cells. The B cells that present Ag more efficiently to T cells (due to higher affinity BCR) get more survival signals through CD40 and cytokines. (Fig. 9.15) Many B cells will have lower affinity, or no change, and will lose competition for Ag and T cell help and will die. Basis for **affinity maturation**.

- GC B cell with high affinity BCR is directed by T cell to undergo differentiation into either plasma cells specialized to secrete antibodies (Fig. 9.11) or **memory B cells** that can respond much more rapidly to antigen.

- Isotype switching also occurs in the germinal center. Isotype switching requires CD40-CD40L interaction and is influenced by cytokines. (Fig. 9.16)

* IL-4, a hallmark of Th2 T cells, induces IgG1 and IgE
* IFN, a hallmark of Th1 T cells, induces IgG2a and IgG3 while inhibiting IgE

-- IgM predominates in primary immune responses, especially early, whereas IgG and other isotypes predominate in later part of primary response and in secondary immune responses

# HyperIgM syndrome

# Patients have higher serum levels of IgM but no IgG or IgA (and no IgE bound to mast cells).

No germinal centers (Fig. 9.17) and little or no SHM.

# X-linked form caused by mutation in **CD40L**---highlights role of T cell help in germinal center formation and CSR and SHM.

# Autosomal recessive form caused by mutation in AID.